Synopsis

Antiurolithiatic Potential of Spermacoce articularis L.f. through

In vivo and In silico Analysis

By

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Urolithiasis, also known as nephrolithiasis or kidney stone disease, is a medical condition characterized by the formation of stones or calculi in the urinary system, affecting the kidneys, ureters, bladder, or urethra of approximately 12-15% of the global population (Romero *et al*. 2010). It is the third most common urinary tract problem worldwide with an increasingly higher recurrence rate. The prevalence of urolithiasis In Asia, reports 5.5-13.4% (Liu e*t al*., 2018), while in India 10-15% (Sofia *et al.* 2016). Demographically, men are 2-3 times more likely to be affected than women (Lin *et al.* 2020), with a peak incidence between 30-60 years. An abnormal increase in calcium, oxalate, and uric acid which reduces urinary citrate levels is the root cause of the formation of stones (Pandhare *et al*. 2021). They form due to an imbalance between water and crystallizable substances in the urine, including minerals such as calcium, oxalate, and uric acid, salts like sodium and potassium, and other compounds like cystine and xanthine. There are four main types of stones: calcium oxalate uric acid, cystine, and struvite stones. Calcium oxalate stones, the most common type, account for approximately 80% of renal stones, struvite stones make up 10%, usually associated with urinary tract infections (UTIs), and form due to bacterial breakdown of urea, leading to increased pH and magnesium ammonium phosphate precipitation. Uric acid stones comprise around 9% of which result from low urine pH, dehydration, and dietary factors, commonly affecting individuals with gout, diabetes, or those consuming a high-protein diet, and 1% of renal stones, occur primarily in individuals with cystinuria, a genetic disorder affecting amino acid reabsorption. Thus, the factors contributing to stone formation include dehydration, concentrated urine, pH imbalance, inadequate clearance, dietary factors like excessive animal protein, sodium, sugar, and oxalate intake, genetic predisposition, and underlying medical conditions like kidney disease, gout, and hyperparathyroidism. The process involves nucleation, where crystallizable substances

combine to form a nucleus, aggregation, where crystals grow and aggregate, and retention, where stones remain in the urinary tract, potentially causing severe pain, obstruction, infection, kidney damage, and recurrent stone formation.

Treatment for urolithiasis, or kidney stones, involves a combination of medical and surgical interventions, depending on stone size, location, and severity. Medical management includes pain management with analgesics, hydration, medical expulsion therapy with alpha-blockers, antibiotics for infected stones or UTIs, and dietary modifications limiting animal protein, sodium, sugar, and oxalate (Daudon *et al*. 2018), Preventive measures focus on increased fluid intake, dietary modifications, potassium citrate supplements for calcium oxalate stones, allopurinol for uric acid stones, and regular follow-up (Assimos *et al*, 2016).

Medicinal plants due to their therapeutic potential, have been used by humans in medicine for decades. Phytomedicine at present is a significant science for both ancient and modern medical systems. Phytochemical investigations are based on the exploration of plants for their use in the production of novel therapeutic drugs. Research on medicinal plants has also led to the discovery of potential novel drugs that are being used to treat a range of illnesses. Many medicinal plants can induce antiurolithiatic effects by many mechanisms which are revealed in previous pharmacological studies. Some medicinal plants exhibit antiurolithiatic properties through their diuretic action, which increases urine production and reduces salt saturation, thereby preventing kidney stone formation. Thus, medicinal plants offer a promising alternative to allopathic treatments for urolithiasis (kidney stones) due to their natural, safe, and cost-effective properties. Unlike allopathic medications, which often have side effects and may not address underlying causes, medicinal plants provide a holistic approach to treatment. Among various traditional medicinal plants employed to combat urolithiasis, *Spermacoce articularis*, a perennial herb, used for centuries

in folk medicine, has demonstrated remarkable efficacy in preventing and treating kidney stones. In Ayurveda *S. articularis* (Fam: Rubiaceae) is known as "Madanaghanti" and in Tamil, it is called "Nathaisuri", is used traditionally to treat eye disorders such as ophthalmia, inflammation of the eye, blindness, sores, and conjunctivitis, and is also used to cure fever, inflammation of gums, and spleen complaints. Leaves extract is used as an astringent in hemorrhoids and gallstones, seeds as a demulcent in diarrhea and dysentery, roots as a mouthwash to cure toothache, and decoction of herb in the treatment of headaches (Maruvoor et al. 2018) The herb has abundant calcium and phosphorus and mainly possesses beta-sitosterol, ursolic acid, iso-rhamnetin, and D-mannitol (Dahiya and Solanki, 2011). Although *S. articularis* is used traditionally there is no report on the antiurolithiatic potential.

Thus, the present study on "Antiurolithiatic Potential of *Spermacoce articularis* L.f. through *In Vivo* and *In Silico* Analysis*"* aims to investigate the antiurolithiatic potential of *S. articularis*, exploring its ability to inhibit calcium oxalate crystal formation, reduce oxidative stress, and urinary risk factors associated with kidney stone formation, to provide scientific evidence for the traditional use of *S. articularis* in treating kidney stones, contributing to developing novel, plant-based therapies for urolithiasis.

The objectives framed for the present investigation were

- ✓ To study the pharmacognostic and phytochemical aspects of different extracts of leaf, stem, and root of *Spermacoce articularis* L.f.
- ✓ To evaluate the total antioxidant efficacy of different solvent extracts of the leaf and stem of *S. articularis* L.f.
- ✓ To explore the therapeutic potential of the leaf and stem of *S. articularis* L.f against urolithiasis

- ✓ To investigate the urolithiasis-inhibiting potential of *S. articularis* L.f stem in the Wistar-albino rat model
- ✓ To evaluate the key bioactive compounds in *S. articularis* L.f stem using TLC and HPTLC.
- ✓ To identify the chemical composition of *S. articularis* L.f stem extract using GC-MS profiling.
- ✓ To validate the binding affinity of bioactive compounds from *S. articularis* L.f stem against urolithiasis receptors.

The organoleptic and fluorescence analysis confirmed the purity and quality of the powdered *S. articularis* samples. The phytochemical analysis of *S. articularis* plant parts revealed that all the solvent extracts of stem and leaf exhibited a significantly higher number of phytochemicals than the root extracts. Quantitative analysis of primary and secondary metabolites showed that stem methanol and leaf ethanol extracts of *S. articularis* possessed the highest concentrations of alkaloids, terpenoids, phenols, and tannins. The highest protein and carbohydrate content was observed with methanol extract of the stem (27.65±0.009 g/100g and 184.44±0.005 mg/g) and leaf (15.64±0.005 g/100g and 152.4±0.032 mg/g). Alkaloid, terpenoid, and tannin content of 71.35± 0.0049 mg/g mg AE/g, 70.42±0.001 mg/g mg linalool/g, and 34.20±0.005 mg/g mg TAE/g respectively was found to be the maximum in stem methanol. The evaluation of enzymatic and non-enzymatic antioxidant capacities in *S. articularis* extracts revealed that the stem methanol extract exhibited significantly higher activity, followed by the leaf ethanol extract, than all other solvent extracts. In the present study methanol extracts of both the leaf and stem samples showed the highest catalase level (2.81 and 5.3.54 units/g). In the case of peroxidase and polyphenol oxidase, the stem sample showed maximum results in methanol extract (1.23 and 2.4.09 units/g). Antioxidant

evaluation of *S. articularis* revealed notable activity, with stem methanol extracts displaying the highest FRAP and ABTS values of 13.84±0.085 and 20.08±0.144 respectively, and leaf ethanol extracts exhibiting the highest DPPH activity 23.55±1.270, indicating its potential as a natural antioxidant. The phytochemical analysis revealed a rich composition of bioactive compounds, particularly in stem and leaf extracts, which exhibited significant antioxidant capacity.

The results indicated that *S. articularis* stem methanol and leaf ethanol extracts possessed anti-crystallization properties, inhibiting CaOx formation in solution with reduced particle size and number at increasing extract concentration (100-500 mg/ml). Stem methanol extract exhibited maximum inhibition of crystal growth and aggregation, compared to ethanol leaf extract, thus stem methanol extract, was selected for subsequent *in vivo* antiurolithiatic studies. The *in vivo* studies demonstrated the anti-urolithiatic potential of *S. articularis* stem methanol (SASM) extract. Treatment with the extract significantly reduced kidney weight and CaOx crystal precipitation compared to the disease control group. Notably, the 500 mg/kg SASM treatment decreased lipid peroxidation and enhanced antioxidant activities, mitigating renal damage. Additionally, the extract reduced urinary biomarkers, including urea, uric acid, and creatinine. Histopathological examination revealed no cellular necrosis or inflammation in the kidney tissues of treated animals, indicating renal protection. Stem methanol extract of *S. articularis* treatment with varying doses (250 mg/kg and 500 mg/kg) suggestively lowered the calcium oxalate crystals deposition, renal tubules dilation, and interstitial inflammation compared to Group II. The rosette-like, polycrystalline CaOx crystals indicated that the particles have adhered to and retained in the renal tubules. On the other hand, stem methanol extract of *S. articularis* treatment significantly decreased the CaOx crystal deposits shown in urine microscopy and

kidney histology. In the normal group, kidney tissues were examined microscopically and no cellular necrosis or inflammatory infiltration was visible in the interstitial space of the kidney tubules. Several inflammatory infiltrations were observed in the renal cortex and renal tubular epithelium of rats injected with PEG. Alternatively, stem methanol extract of *S. articularis* treatment reduced the large range of infiltrates. These findings suggest that *S. articularis* stem methanol extract exhibits protective effects against CaOx urolithiasis. The cytotoxicity evaluation of *S. articularis* stem methanolic extract using the brine shrimp larvae assay revealed a favorable safety profile, with low toxicity observed at both lower and higher concentrations. The extract demonstrated an LC50 value of 180.00 µg/ml, indicating its potential as a safe therapeutic agent. These findings suggest that *S. articularis* stem methanolic extract may be considered for further development as a therapeutic agent, owing to its minimal cytotoxic effects. The stem methanol extract showed exceptional anti-crystallization properties, inhibiting calcium oxalate formation and displaying renal protective effects *in vivo*.

TLC and HPTLC analysis revealed the presence of terpenoids and phenols in the SASM, consistent with literature reports. HPTLC analysis detected terpenoids (16.96 µg/mL) and phenols (13.46 µg/mL) in SASM at 3mg/mL, confirming the presence of these phytoconstituents. The GC-MS profile detected 43 phytocompounds, including 29 organic compounds from various functional groups, which were differentiated based on their retention times. The major compounds catechol, vanillin, gamma-tocopherol, eugenol, and vitamin E were found to be present in SASM.

Twenty-nine bioactive compounds were selected against three key proteins involved in maintaining renal health such as Tamm-Horsfall Protein (THP), Calcitonin, and Calcium Oxidoreductase. Among them, D-mannitol demonstrated a high interaction of -6.406

kcal/mol with Tamm-Horsfall Protein, -8.41 kcal/mol with calcitonin, and -7.289 kcal/mol with calcium oxidoreductase. MD simulations indicated that D-mannitol may effectively inhibit calcitonin hormone, vital for maintaining kidney function. D-mannitol formed stable interactions with key amino acids in calcitonin via both hydrogen bonding and hydrophobic interactions. D-mannitol showed promise as a potential anti-urolithiasis agent by inhibiting calcitonin hormone, which requires further investigation to confirm its efficacy and safety. The identified bioactive compounds showed favorable pharmacokinetic properties with minimal violations of Lipinski's rule. The identification of 29 bioactive compounds, including organic acids, fatty acids, triterpenes, and phenolic compounds, underscores the plant's therapeutic potential. D- Mannitol also interacted through the strong hydrogen bonds with the crucial amino acids in Calcitonin hormone, such as ARG 58, CYS 81, ASP 83, ASP 84, THR 85, SER 91, TYR 92, GLN 93 of chain A and TYR 41, VAL 42, ARG 45, LYS 46 of chain B. Hydrophobic interactions are seen among the residues TRP 82 chain A, VAL 42, MET 49 of chain B. D- Mannitol formed strong hydrogen bonds and hydrophobic interactions with the amino acids present in the catalytic domain of the Calcitonin hormone. Ligand RMSF depicted how the ligand fragments interacted with protein, and 12 heavy atoms in D-Mannitol were found to be present. Specifically, D-mannitol exhibited strong anti-urolithiasis properties through molecular docking, highlighting its promise in treating kidney stones.

The phytochemical analysis revealed a rich composition of bioactive compounds, particularly in stem and leaf extracts, which exhibited significant antioxidant capacity. The stem methanol extract showed exceptional anti-crystallization properties, inhibiting calcium oxalate formation and displaying renal protective effects *in vivo*. The identification of bioactive compounds, including organic acids, fatty acids, triterpenes, and phenolic

compounds, underscores the plant's therapeutic potential. Specifically, D-mannitol exhibited strong anti-urolithiasis properties through molecular docking, highlighting its promise in preventing and treating kidney stones. The safety profile of *Spermacoce articularis* stem methanol extract, as evidenced by its low toxicity (LC50: 180.00 µg/ml), supports its potential as a safe and effective therapeutic agent against calcium oxalate urolithiasis. Overall, these findings suggest that *Spermacoce articularis* is a valuable natural resource for the development of novel antioxidant and anti-urolithiasis treatments, warranting further investigation and clinical translation.

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